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Homeostatic regulation of supercoiling sensitivity coordinates transcription of the bacterial genome

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Regulation of cellular growth implies spatiotemporally coordinated programmes of gene transcription. A central question, therefore, is how global transcription is coordinated in the genome. The growth of the unicellular organism *Escherichia coli* is associated with changes in both the global superhelicity modulated by cellular topoisomerase activity and the relative proportions of the abundant DNA-architectural chromatin proteins. Using a DNA-microarray-based approach that combines mutations in the genes of two important chromatin proteins with induced changes of DNA superhelicity, we demonstrate that genomic transcription is tightly associated with the spatial distribution of supercoiling sensitivity, which in turn depends on chromatin proteins. We further demonstrate that essential metabolic pathways involved in the maintenance of growth respond distinctly to changes of superhelicity. We infer that a homeostatic mechanism organizing the supercoiling sensitivity is coordinating the growth-phase-dependent transcription of the genome.

Keywords: FIS; H-NS; supercoiling; transcription regulation; metabolism

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INTRODUCTION

Understanding the mechanisms of concerted rearrangements of gene activities during growth and development is a fundamental problem. How is genomic expression organized in a cell and is there a common coordinating mechanism? Almost five decades ago, John von Neumann proposed that the flow of genetic information is coordinated by a specific relationship between the 'digital' (discontinuous) properties of unique genes and 'analog' (continuous) properties of the gene products (von Neumann, 1958). Such a relationship evidently develops during pattern formation in *Drosophila* embryogenesis, in which the essentially 'analog' information provided by concentration gradients of transcription factors is converted into 'digital' patterns of transcripts by means of differential protein interactions occurring on spatially separated transcriptional enhancers (Sauer *et al*, 1996). Most clearly, this fundamental principle can be demonstrated in *Escherichia coli*, a classical model organism that shows strict correlations between the global gene expression patterns and different growth states (Tao *et al*, 1999; Wei *et al*, 2001; Weber *et al*, 2005) and also enables the modulation of genomic transcription by alterations of global DNA superhelicity (Jeong *et al*, 2004; Peter *et al*, 2004; Willenbrock & Ussery, 2004; Travers & Muskhelishvili, 2005a). Such alterations are associated with both growth transitions and stress responses to environmental challenge, supporting the idea that DNA supercoiling itself might act as a principal coordinator of global gene expression (Balke & Gralla, 1987; Dorman, 1996; Tse-Dinh *et al*, 1997; Cheung *et al*, 2003; Travers & Muskhelishvili, 2005b). Nevertheless, as less than 8% of specific genes are found to respond to supercoiling in *E. coli* (Peter *et al*, 2004), the role of superhelicity in organizing the global growth-phase-dependent transcription remains obscure.

Previous studies proposed that binding of abundant chromatin proteins could selectively direct the supercoiling energy to gene promoters (Travers & Muskhelishvili, 1998; Hatfield & Benham, 2002; Muskhelishvili & Travers, 2003). In this study, we investigated the transcriptional effects of two such proteins, factor for inversion stimulation (FIS) and histone-like nucleoid structuring protein (H-NS), which act as global pleiotropic regulators in *E. coli*. H-NS can activate transcription but is predominantly a universal repressor for the bacterial genome, whereas FIS modulates the transcription of many genes implicated in regulation of metabolism and growth (Gonzalez-Gil *et al*, 1996; Dorman, 2004; Kelly *et al*, 2004; Rimsky, 2004). Previously, it was shown that the loss of H-NS and FIS affects the superhelical density of plasmid DNA (Owen-Hughes *et al*, 1992; Schneider *et al*, 1997). By combining the effects of mutations in the *fis* and *hns* genes with experimentally induced changes of global superhelicity, we demonstrate here that the organization of global transcription is tightly coupled to distribution of supercoiling sensitivity in the genome.

RESULTS AND DISCUSSION

FIS and H-NS are present in several tens of thousands of copies per cell and can both activate and repress many genes by direct

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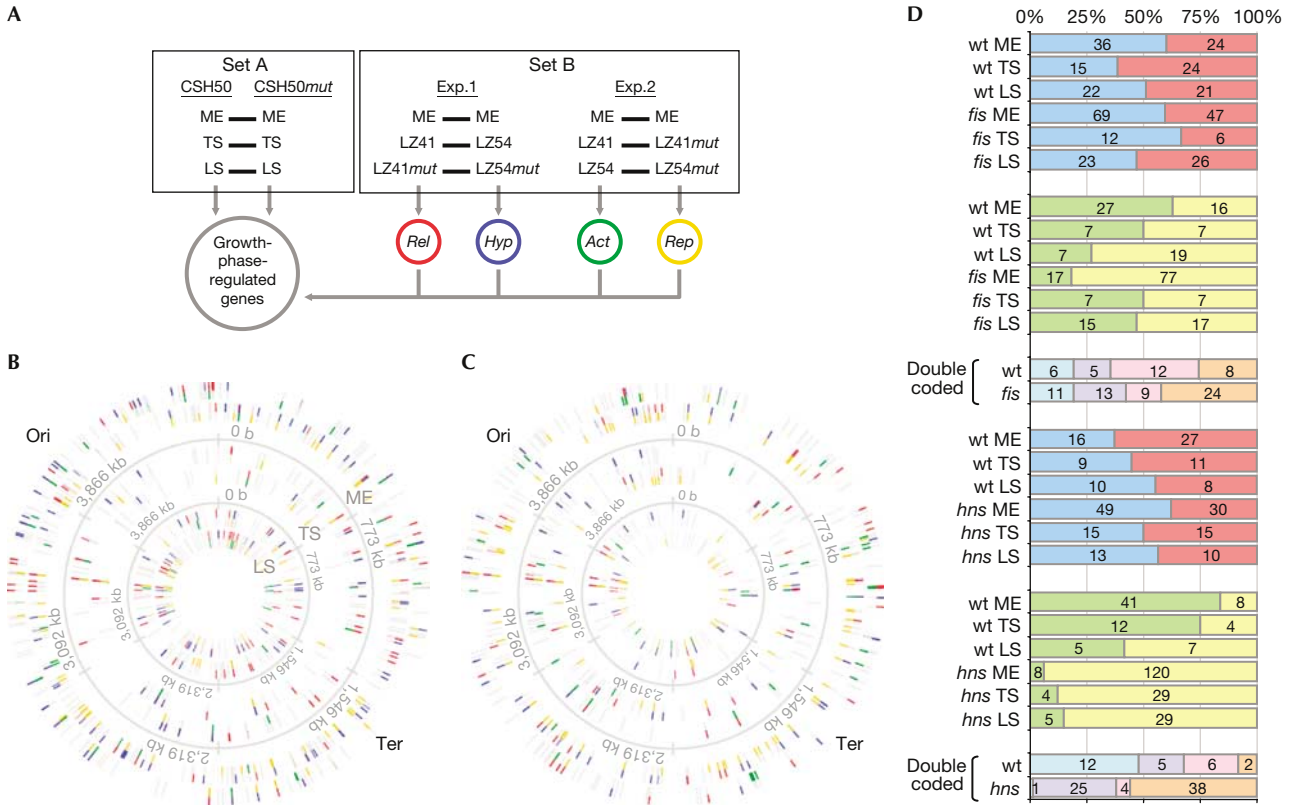

Fig 1 | Strategy of mapping supercoiling-associated genes. (**A**) Set A compares the transcript profiles of the CSH50 wild-type and mutant cells during growth. Set B compares the transcript profiles of LZ41 and LZ54 strains and their derivatives after norfloxacin treatment. (**B**) Genomic wheels showing spatiotemporal distributions of *Rel* (red), *Hyp* (blue), *Act* (green) and *Rep* (yellow) transcripts among the growth-phase-dependent genes (grey) in CSH50 wild type (outer wheels) and *fis* (inner wheels). (**C**) Transcript distributions in CSH50 wild type and *hns* mutant. (**D**) Changes of supercoiling-associated genes during growth. Distributions of *Hyp* (blue), *Rel* (red), *Act* (green) and *Rep* (yellow) gene transcripts are shown. The sums of total mapped *Hyp* + *Rel* or *Act* + *Rep* transcripts were set at 100% for each growth phase. The distinct 'double-coded' transcripts—*Hyp/Act*, *Hyp/Rep*, *Rel/Act* and *Rel/Rep*—are ordered from left to right for each bar. The sum of total mapped double-coded transcripts for the entire growth cycle was set at 100%. The number of genes is indicated within the bars. *fis*, CSH50 *fis*; *hns*, CSH50 *hns*; LS, late stationary phase; ME, mid-exponential phase; Ori, origin; Ter, terminus of replication; TS, transition state; wt, CSH50 wild type.

binding (Dorman & Deighan, 2003). However, FIS forms a concentration gradient decreasing by two orders of magnitude on transition to the stationary phase, whereas the concentration of H-NS remains relatively constant (Ball *et al*, 1992; Azam & Ishihama, 1999). As both these regulators modulate supercoiling, we expected to observe both direct and indirect effects on global transcription during growth. We used a novel DNA microarray-based strategy to link the growth-phase-dependent transcription of genes with supercoiling sensitivity. In one set of experiments, we compared the growth-phase-dependent transcript profiles of the wild type with both the *fis* and *hns* mutants to distinguish between the genes expressed in the presence or absence of each regulator (Fig 1A, data set A). In the second set of experiments (data set B), we used genetically engineered *E. coli* LZ41 and LZ54 strains containing drug-resistant topoisomerase gene alleles to inhibit DNA gyrase or topoisomerase IV activity selectively and thereby induce relaxation or high negative supercoiling, respectively (Zechiedrich *et al*, 1997). On introduction of the *fis* and *hns* mutations in the LZ41 and LZ54 strains, the global supercoiling response to drug addition was not substantially altered ($\sigma = -0.082$ for LZ54 wild type and -0.086 for both LZ54*fis* and LZ54*hns* mutants; $\sigma = < -0.031$ for both LZ41 wild type and mutants; supplementary Fig 2 online). Thus, using distinct combinations of the LZ41 and LZ54 strains and their mutant derivatives, we could vary superhelical density to distinguish gene transcripts associated with either relaxation (*Rel*) or high negative supercoiling (*Hyp*) in each genetic background (Fig 1A, Exp.1; the term 'associated' emphasizes the difficulty of identifying whether these transcripts are activated by a given supercoiling regime or inactivated by the opposite one) and also those either activated (*Act*) or repressed (*Rep*) by each regulator at various superhelicities (Exp.2*;* we note that the regulator effects can be either direct or indirect). These 'supercoiling-associated' transcripts were then mapped on the growth-phase-dependent transcript profiles of data set A to evaluate the input of supercoiling sensitivity in the organization of global transcription.

Supercoiling sensitivity of global transcription

The results of mapping are presented on genomic wheels in Fig 1B,C. About 50% of all transcripts could be mapped (supplementary Table III online), indicating that these variable patterns are largely organized by supercoiling-associated genes. Notably, out of a total of 1,596 *Hyp* and *Rel* transcripts identified, only 145 were common to the wild-type, *fis* and *hns* backgrounds, indicating that these supercoiling response depends on the genetic context. By using *fis* and *hns* mutants, we thus greatly expanded the repertoire of supercoiling-associated transcripts identified. The observed interspersed pattern of the domains of relaxation and high negative superhelicity agrees with both the formation of fluid short-range domains of distinct topologies in the *E. coli* chromosome (Postow *et al*, 2004; Stein *et al*, 2005) and the complex dependence of long-range transcriptional patterns on supercoiling (Jeong *et al*, 2004). This supports the idea that transcription is governed by 'effective' superhelicity—a parameter reflecting the dynamic nature of competition between topoisomerases, transcription machinery and chromatin proteins for available supercoils (Travers & Muskhelishvili, 2005b). Variable distributions of supercoiling sensitivity reflect a compound effect of several cooperating factors in addition to FIS and H-NS, including all chromatin architectural proteins, more dedicated transcriptional regulators and perhaps the chromosome partitioning proteins (Malik *et al*, 1996; Sawitzke & Austin, 2000; Beloin *et al*, 2003; Frenkiel-Krispin *et al*, 2004). However, our data show that the lack of a single key component of bacterial chromatin—either FIS or H-NS—can reorganize the supercoiling sensitivity of genomic transcription, consistent with the previously proposed global homeostatic feedback (Schneider *et al*, 2000). As expected from such a mechanism, all strains grew with comparable rates, despite remarkable variations of transcript patterns (supplementary Fig 1 online). Nevertheless, during prolonged growth, mutations in *fis* can confer a competitive advantage or disadvantage depending on the precise environment (Schneider *et al*, 1997; Crozat *et al*, 2005).

Variations of supercoiling-associated transcripts

Distributions of supercoiling-associated transcripts in the wild-type, *fis* and *hns* cells varied with growth. The exponentially growing *fis* mutant showed an increase of both the *Hyp* and *Rel* transcripts compared with those in wild type, whereas in the *hns* mutant the relative proportion of *Hyp* transcripts increased, in keeping with in the observed increase of global negative superhelicity (Fig 1D; supplementary Fig 2B,C online). Furthermore, on transition to the stationary phase, a lower proportion of *Rel* genes were observed in the *fis* mutant than in the wild type, but not in the stationary phase itself. As expected, the effect of *hns* mutation was most pronounced for the *Rep* genes. In the *fis* mutant, the *Rep* genes increased only during the exponential phase, whereas in the *hns* mutant, the *Rep* genes predominated during the entire growth cycle. Also the 'double-coded' transcripts responding to specific combinations of supercoiling and genetic background were enriched for *Rep/Hyp* and *Rep/Rel* genes, especially in *hns* cells. Taken together, these observations are in excellent agreement with differential abundance of FIS and H-NS during the growth cycle and the general repressor role of H-NS (Ball *et al*, 1992; Dorman, 2004).

Table 1 | Supercoiling sensitivity of genes of biosynthesis and degradation pathways* expressed in the wild-type and mutant strains

	Biosynthesis (*Hyp*/*Rel*)	Degradation (*Hyp*/*Rel*)
Wild type	4.29	0.54
fis	2.07	0.87
hns	1.91	1.0

*The metabolic pathways comprising 421 genes for biosynthesis and 223 genes for degradation were derived from http://EcoCyc.org. The *Hyp* to *Rel* transcript ratios are derived from the data set B for 148, 111 and 134 metabolic genes expressed in the wild type, *fis* mutant and *hns* mutant, respectively.

Supercoiling sensitivity is coupled to metabolic function

Interestingly, the analysis of distribution of *Hyp* and *Rel* genes among the functional groups involved in essential cellular metabolism demonstrated a higher proportion of *Hyp* genes in the anabolic than in the catabolic pathways, which was especially remarkable in the wild-type cells (Table 1). Analysis of a pathway of exceptional importance for vitality—the citric acid cycle—demonstrated that the crucial steps producing combustible fuel in the form of reducing equivalents NADH and $FADH_2$, which are required for generation of ATP by oxidative phosphorylation, are associated with DNA relaxation. In contrast, when we examined the glyoxalate bypass, topping up the cycle and increasing the net synthesis of carbohydrate, we found this pathway to be associated with high negative superhelicity (Fig 2A; supplementary Table II online). This suggests that high ATP/ADP ratios favouring supercoiling of DNA by gyrase would facilitate glyoxalate bypass and plastic substrate synthesis, whereas DNA relaxation would favour the production of ATP required to maintain gyrase activity. Furthermore, we found that the *de novo* pathway of nucleotide biosynthesis explicitly involves the *Hyp* genes (Fig 2B). Most of these genes have promoters with a GC-rich 'discriminator' sequence, which confers sensitivity to both high negative supercoiling and the regulatory nucleotide ppGpp (Cashel, 1970; Zalkin & Nygaard, 1996; Figueroa-Bossi *et al*, 1998). Notably, both the superhelicity and ppGpp concentration are elevated in *fis* cells (Travers & Muskhelishvili, 2005b), indicating a homeostatic regulation mechanism. The observed coupling of distinct supercoiling sensitivities to essential metabolic pathways provides new insights into the mechanisms that coordinate central metabolism, and also sheds light on the puzzling observation that mutations in metabolic genes can affect DNA topology (Hardy & Cozzarelli, 2005). An important future task is the clarification of mechanistic effects of changes in superhelicity on transcription, which could modulate not only transcription initiation but also elongation and termination.

Our mapped transcript profiles describe the distributions of supercoiling sensitivity during growth, rather than the strength of transcriptional response to superhelicity and are consistent with the proposed role of FIS and H-NS in forming topological barriers on the chromosome (Hardy & Cozzarelli, 2005; Dame, 2005). However, Hardy & Cozzarelli failed to detect any alterations of plasmid supercoiling in *fis* and *hns* mutants, most probably because they did not analyse the entire growth cycle, which is necessary to show the dynamic changes of superhelicity (supplementary Fig 2B,C online; Schneider *et al*,

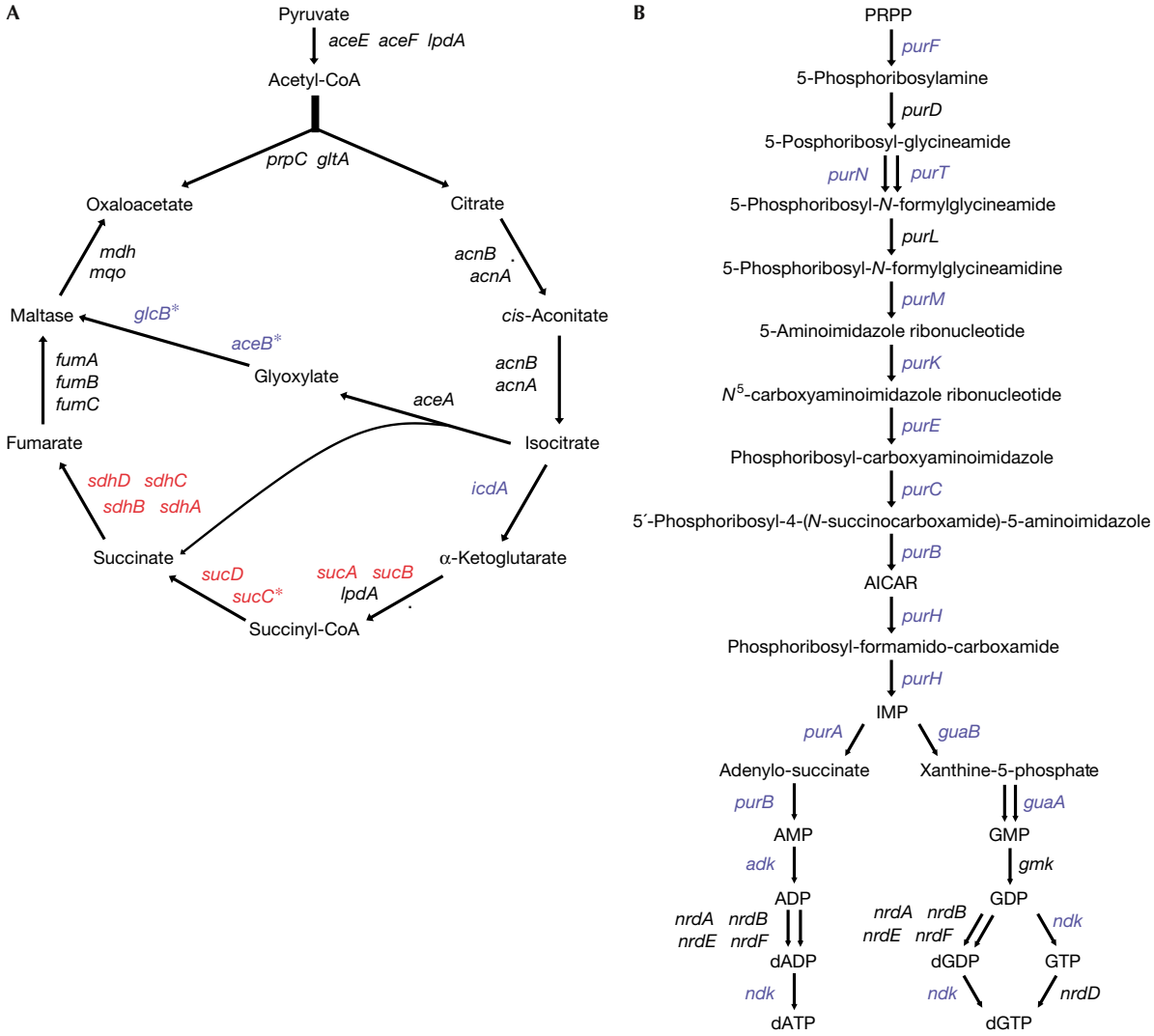

Fig 2 | Coordination of essential metabolic pathways by supercoiling sensitivity. (**A**) Regulation of oxaloacetate production in citric acid cycle and glyoxalate bypass. The *Rel* genes are in red letters and the *Hyp* genes are in blue. Note that the citric acid cycle involves *Rel* genes, whereas the glyoxalate bypass involves *Hyp* genes. (**B**) Coordination of *de novo* biosynthesis of purine nucleotides. Note that almost all the steps of biosynthesis from phosphoribosylpyrophosphate (PRPP) involve *Hyp* genes. The pathways were derived from http://ecocyc.org. The genes identified by Real-Time PCR are marked with asterisks.

1997). Despite substantial differences in the experimental design and assay conditions, we identified the supercoiling sensitivity of previously described genes (Peter *et al*, 2004). Also, the functional gene groups discovered in this study are consistent with those reported for a *fis* mutant of *Salmonella typhimurium* and *hns* mutant of *E. coli* (Hommais *et al*, 2001; Kelly *et al*, 2004).

We thus infer that the coordination of growth-phase-dependent transcription involves a homeostatic mechanism that organizes the supercoiling sensitivity in the genome. As proposed in our previous work (Schneider *et al*, 1999, 2000), this feedback implicates chromatin proteins that constrain DNA supercoils and act as 'optimizers' of cellular metabolism. Whereas the metabolic status determines the overall supercoiling level (van Workum *et al*, 1996), we show here that the genomic distributions of superhelicity can specify the patterns of transcripts during growth. Global regulation thus seems to be a genuine device converting the 'analog' information of torsional energy distributions into 'digital' patterns of responding genes. Our observations provide a holistic conceptual framework for analysis of global transcription and reinforce the notion of John von Neumann of coordination of information flow in the genetic system. We note that DNA supercoiling is implicated in the regulation of eukaryotic transcription (Mizutani *et al*, 1991; Dunaway & Ostrander, 1993; Caserta & Di Mauro, 1996; Kouzine *et al*, 2004). Thus, studies of regulated alterations of DNA superhelicity might be essential for understanding the coordinated gene functions in eukaryotes as well.

METHODS

DNA microarray analysis. The *E. coli* K12 strains used in this study for RNA isolation are described elsewhere (Zechiedrich *et al*, 1997). The *fis* and *hns* mutants of LZ54 and LZ41 strains were obtained by P1 phage transduction. The transcript profiling for the LZ strains was carried out after brief (5 min) treatment of cells growing exponentially in 2 × YT medium at 30 °C with moderate concentrations of norfloxacin (20 μg/ml). All other strains were grown in 2 × YT medium at 37 °C. DNA microarray experiments were performed according to OciChip™-Application Guide (http://www.ocimumbio.com) as two biological replicates with two technical replicates each (except for the transition state of CSH50*fis* and the stationary phase of CSH50*hns* strains; supplementary information online). Scanned array images were quantified and normalized using the TM4 software package. A one-class *t*-test was applied to replicated experiments to obtain genes with significant *P*-values ($P<0.05$). Further details are provided in the supplementary information online.

Real-time PCR. QuantiTect® SYBR® Green one-step Real-Time PCR reactions (Qiagen GmbH, Hilden, Germany) were performed in triplicate, following the manual of the manufacturer and using an Mx3000P™ Real-Time cycler (Stratagene®, La Jolla, CA, USA).

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

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Array Express accession number: I-TABM-86.

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